

Immunomodulator effects of ultra high dilutions of *Gelsemium sempervirens* L., Poumon histamine and Histaminum in stressed Mice

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Abstract

This work reports short term of stress immune-related abnormalities in control and treated mice with three homeopathic drugs. The aim of this study was to evaluate the effects of ultra high dilutions of *Gelsemium sempervirens* L., Poumon histamine and Histaminum on stress-induced cellular immune disturbances. Immunological studies were investigated to count the lymphocytes subpopulations T helper CD4+, T cytotoxic /suppressor CD8+, Natural Killer cells (NK), by flow cytometry (Facsan). These results have demonstrated that low dilutions (9 CH) of *G. sempervirens*, Poumon histamine and Histaminum, showed a significant immunoprotective like effect on regulation of natural killer cells in stressed mice. Whereas the high dilutions of these three drugs (15 and 30 CH) showed a significant immunostimulant like effect on regulation of NK cells in stressed mice. Immune system changes may account for the relationship between stress and immune disease. In this work, we propose the "stress immune" model as a biological pathway to explain the immunomodulator pharmacological properties of the homeopathic drugs studied.

Key words: Stress, *Gelsemium sempervirens* L., Poumon histamine, Histaminum, Immunoprotective like effect, Immunostimulant like effect.

Introduction

Stress is interpreted by the body as a danger signal which affects immune system (Ader *et al.*, 2001). It had been demonstrated that acute stress may result in a suppression of immune activity (Dantzer & Kelly 1989, Millan *et al.* 1996, Marsland *et al.* 1997, Vuitton *et al.* 1999, Maddock & Pariente 2001). It's known that the evolution from health to disease could be associated, at least partially, with a 'passive' immunosuppressive mode of response, mediated by the pituitary-adrenal axis, typically the opposite of an 'active,' immunostimulant mode of response, mediated by adrenergic stimulation. The glucocorticoids have a direct effect on

the number of T cells, increased the Th2 response (Tamada *et al.*, 1998). Whereas, when catecholaminergic system is activated after stress exposure, it induced an increase of T cells proliferation and cytokines production (Sanders, 1998). There are other neuromediators as CRH, ACTH and β endorphins, which induced an immunosuppressive effect (Reul *et al.*, 1998). The work of Brydon *et al.* (2005), demonstrated that psychological stress activates interleukin-1 β gene expression (inflammatory cytokine), which is a novel mechanism potentially linking stress and heart disease.

In this present study, we used the "stress immune" model as a biological

pathway to explain the immunomodulator properties of

Concerning *G. sempervirens*, it has reported a multiple animal neurological intoxications associated with this species (Thompson *et al.*, 2002). Others authors have been isolated a several derivatives from a MeOH extract of the stem of *G. sempervirens* and founded to be the principal cytotoxic entities (Schun & Cordell, 1987). Furthemore, the gelsedine type oxindole alkaloids, were isolated from the stems and leaves of cultivated *G. sempervirens* and their structures were detected by spectroscopic analysis (Kitajima *et al.*, 2003).

The *P. histamine* and Histaminum contains several inflammatory and allergic mediators such as histamine, leukotriene., and regulates allergic reaction mechanisms. The clinical studies showed that *P. histamine* was indicated in the regulation of the

Gelsemium sempervirens L., Poumon histamine and Histaminum.

mechanism of allergic reaction and especially for the respiratory ways. Experimental research confirmed the action of high dilutions of *P. histamine* and Histaminum on the mechanism of allergic reaction, in particular on the modulation of activation of human basophiles degranulation. These studies demonstrated that the experimental activity of *P. histamine* is superior to Histaminum, due to a complex mixture of *P. histamine* (Demarque *et al.*, 1995).

In this work, we seek the immunoprotective and immunostimulant properties of *G. sempervirens*, *P. histamine*, and Histaminum that can be developed as candidates for treatment of immunodepressive symptoms of some of immune diseases or in some cancer diseases.

Materials and methods

Animals

Male Swiss mice (Breeding R. Janvier, France), weighing between 38 and 44 g-12 weeks of age, were used in all experiments. Mice were acclimatized to laboratory conditions for 14 days before being used. During this time animals were group-housed (five per box) in polypropylene boxes containing food and water ad libitum. The light cycle was automatically controlled (12h light/12h darkness) and room temperature adjusted to maintain $22 \pm 2^\circ\text{C}$.

Preparation of products

G. sempervirens, *P. histamine* and Histaminum were obtained from Boiron laboratories. The dilutions of 9, 15, 30 CH "Centesimal Hahnemanienne", were prepared by centesimal dilution 1/100 (1ml of product /99 ml of alcohol) and injected intraperitoneally (ip) 30 min before stress exposure (n=10). Two control groups (unstressed) were treated

by an equal volume of these products or by NaCl 0.9 %.

The preparations used in these experiments were:

- *P. histamine*: it's prepared from the lung of guinea pig sacrificed during anaphylactic shock provoked artificially with ovalbumine,
- Histaminum: it's prepared by dilutions of histamine chlorydrate; it is especially used for cutaneous allergic reactions, but the lung histamine remains the most medicine used in the allergic illnesses, even in the cutaneous indications.
- *G. sempervirens* L.: it's prepared from a climbing shrub of *Loganiaceae* family. The dyeing mother is essentially prepared from the root of the plant.
Common Name: Carolina jessamine
Family: *Loganiaceae*

Country of Origin: South-eastern N. America - Florida to Texas, N to Arkansas & W. Virginia
 Habitat: Along sea coasts
 Uses: This plant has numerous therapeutic uses, although extreme care should be taken as all parts of the plant are considered toxic and potentially fatal if ingested.

Procedure of stress

Previous studies in our laboratory have indicated the procedure of stress (Boustia *et al.*, 2001). The control groups were composed by two groups: the first group received NaCl 0.9 % without stress; the second group received different drugs 30 min before stress.

The stressed groups were composed also by two groups: the first group received NaCl 0.9 % 30 min before stress exposure, the second group received products 30 min before stress. All the animals were sacrificed 24 h after stress application. Time separating application of stress and animal sacrifice was kept constant (24 h).

Measurement of plasmatic concentration of "stress hormones"

In the objective to confirm the stress situation, we carried out the measurement of plasmatic concentration of corticosterone, epinephrine and

norepinephrine by RIA and HPLC techniques respectively.

Histological cuts

We proceeded to histological cuts with systemic standard coloration (Hematoxyline Eosine), to evaluate the impact of stress on the adrenal fabric, site of production of catecholamine and corticosterone.

Immunological studies

- Blood collection:

Mice were anesthetized with an i.p injection of the pentobarbital (Sanofi, France), 6% diluted at 1/10 in the NaCl 0.9 %. Blood was then collected in heparinized tubes by heart puncture one day following stress exposure.

- Percentage of subpopulations lymphocytes:

Percentage of subpopulations lymphocytes in peripheral blood (TCD4, TCD8 and NK cells) was determined by flow cytometer (FACS).

Statistical analysis

Data are expressed as means \pm S.E.M and statistical differences were calculated by One Way ANOVA-two factors and *t*-test (comparison per pair). Differences between means were considered significant if $p < 0.05$.

Results

Effects of experimental stress on plasmatic concentration of "stress hormones": corticosterone, epinephrine and norepinephrine

As shown in figure 1 and table 1, the experimental stress induced an increase in plasmatic "stress hormones". We noted a significant increase in plasmatic concentration of corticosterone ($p = 0.0006$), a moderate increase in plasmatic concentration of epinephrine and a strong increase of norepinephrine in stressed mice (respectively: $p = 0.008$; $p = 0.003$).

Effects of experimental stress on adrenal fabric (site of production of catecholamine and corticosterone)

The adrenal gland of stressed mice (b) compared to control (a), presents a vascular dilatation, a little marked increase at adrenal medulla volume with an infiltration of some polynuclear cells.

Pharmacological effects of ultra high dilutions of *G. sempervirens*, Poumon histamine and Histaminum on cellular immune response in stressed mice

- *G. sempervirens*

As shown in figure 2a, *G. sempervirens* at 9 and 15 CH, induced a significant increase in T CD4 number in stressed mice (respectively: $p=0.022$;

$p=0.007$). However, the unstressed and treated mice with all dilutions, showed a reduction in TCD4 number (respectively: $p=0.017$; $p=0.015$, $p=0.0004$).

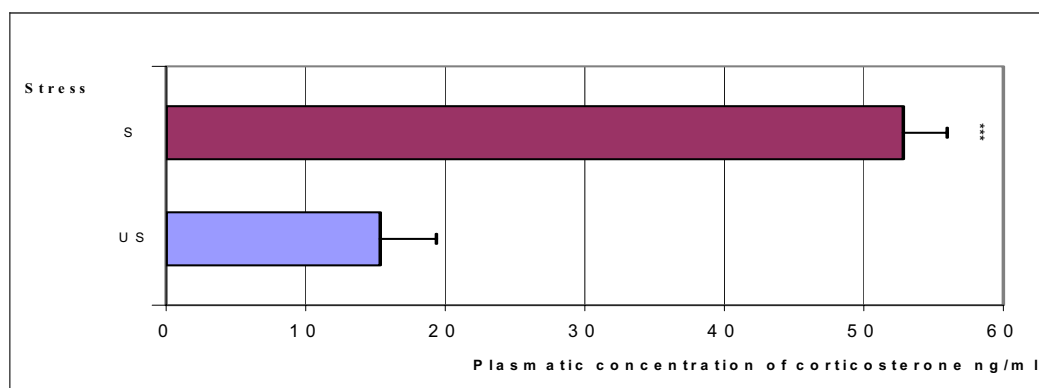


Figure 1. Effect of experimental stress on plasmatic concentration of corticosterone in mice. N= 14.
***: $P < 0.001$.

Table 1. Effect of experimental stress on plasmatic concentration of catecholamines in mice. N= 10.

	Number of animals	Epinephrine (pg/ml) mean \pm SEM	Norepinephrine (pg/ml) mean \pm SEM
Unstressed	5	15.860 \pm 0.940	18.310 \pm 1.540
Stressed	5	21.560 \pm 1.460 t -test=-4.096, P= 0.008	61.500 \pm 14.900 t -test=-4.289, P= 0.003

As indicated in figure 2b, *G. sempervirens* at 15 and 30 CH, induced a significant reduction in TCD8 number in stressed groups, compared to stressed saline groups (respectively, $p=0.004$; $p=0.003$). Whereas, we have noted any significant variation on TCD8 number in unstressed groups.

As shown in figure 2c, *G. sempervirens* at 15 and 30 CH, caused a significant increase in the number of NK cells (respectively, $p=0.006$, $p=0.03$) in stressed groups. Whereas 9 CH has rather tendency to reverse the stress-induced effects, by decreasing the NK cells number ($p=0.025$). However, the unstressed groups treated with the same dilution (9 CH), rather tended to increase

significantly the NK cells number. Consequently, we supposed that *G. sempervirens* acted differently as “anti-stress” or “pro-stress” according to dilutions and to presence or not of stress.

- Poumon histamine

According to figure 3a, the treatment at all dilutions of P. histamine increased the number of TCD4 cells in stressed groups (respectively $p=0.009$, $p=0.006$, $p=0.0018$). However, the number of TCD4 cells in the unstressed groups, rather induced a decrease after a treatment with all dilutions 9, 15 and 30 CH (respectively $p=0.013$, $p=0.006$, $p=0.002$).

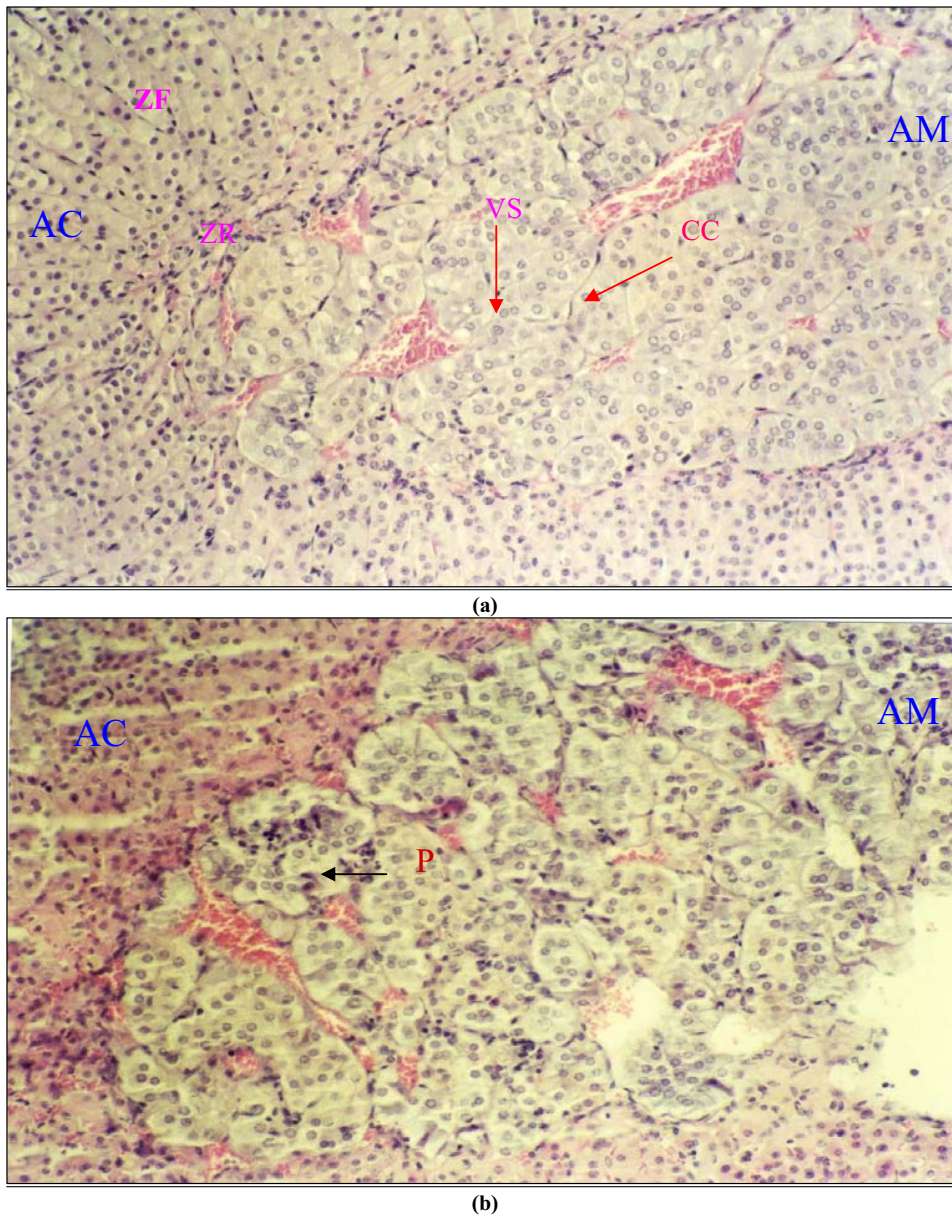


Photo 1. Histological cuts of adrenal gland of unstressed mice (a) and exposed to an experimental stress (b). X 40. AC: Adrenal Cortex; AM: Adrenal Medulla. ZF: Fasciculate Zone; ZR: Reticulate Zone; CC: Chromaffine Cells. VS: Secretion Vesicles. P: Polynuclear.

The results of the figure 3b showed that P. histamine 15 CH, induced a reduction on T CD8 number in both stressed ($p=0.002$) and unstressed groups ($p=0.04$).

As indicated in figure 3c, the treatment of unstressed groups by 9 CH of P. histamine, increased the number of NK cells ($p=0.003$). However, 9 CH has tendency to reverse the effect of stress on regulation of NK cells in stressed group

($p=0.002$). Whereas high dilutions (15 and 30 CH) induced rather an important increase of NK cells number (respectively $p=0.0013$; and $p=0.0004$) compared to stressed-saline groups. We can suppose that 9 CH of P. histamine acted as “anti-stress or immunoprotective”, whereas 15 and 30 CH induced an immunostimulation effect.

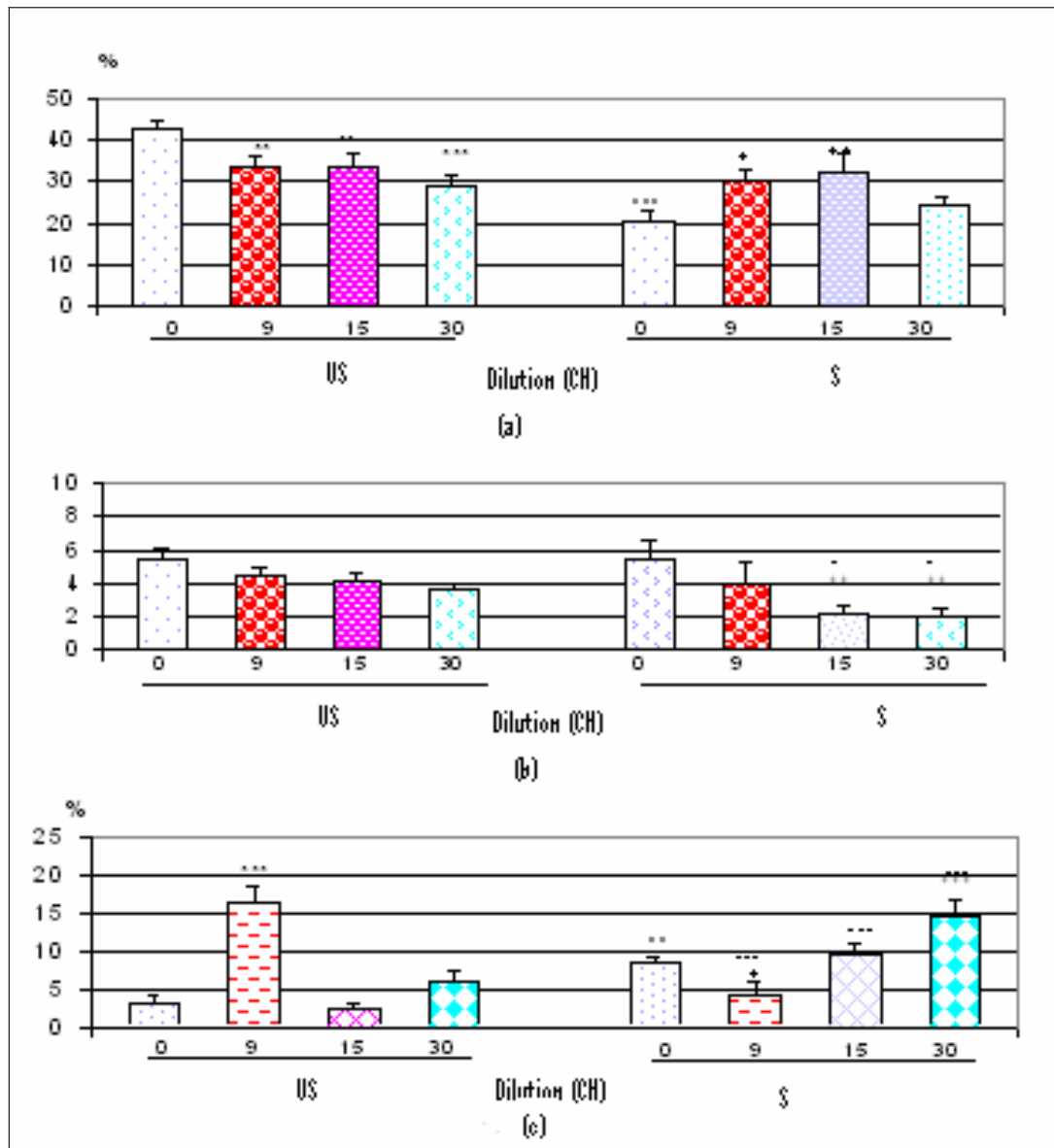


Figure 2. Effects of high dilutions of *G. sempervirens* on percentage of TCD4 (a), TCD8 (b) and NK (c) cells in mice. N=216. US: Unstressed, S: Stressed. CH: Centesimal Hahnemanienne. (a)[°]: US0/S0, * : US0/USGel9,15,30; + : S0/SGel9,15. (b) : + : S0/Gel15et30; - : USGel15,30/SGel15,30. (c)[°]: US0/S0, * : US0/USGel9, + : S0/SGel9,30, - : USGel9,15,30/SGel9,15,30.

- Histaminum

As shown in figure 4a, the treatment of stressed groups by Histaminum 9 and 15 CH, increased the number of TCD4 lymphocytes (respectively, $p=0.03$, $p=0.0004$). Whereas, 9 and 30 CH tended to reduce the number of TCD4 cells in unstressed

groups (respectively $p=0.004$ and $p=0.0007$).

No significant statistical variation has shown, in both, unstressed and stressed treated groups at all dilutions of Histaminum (Figure 4b).

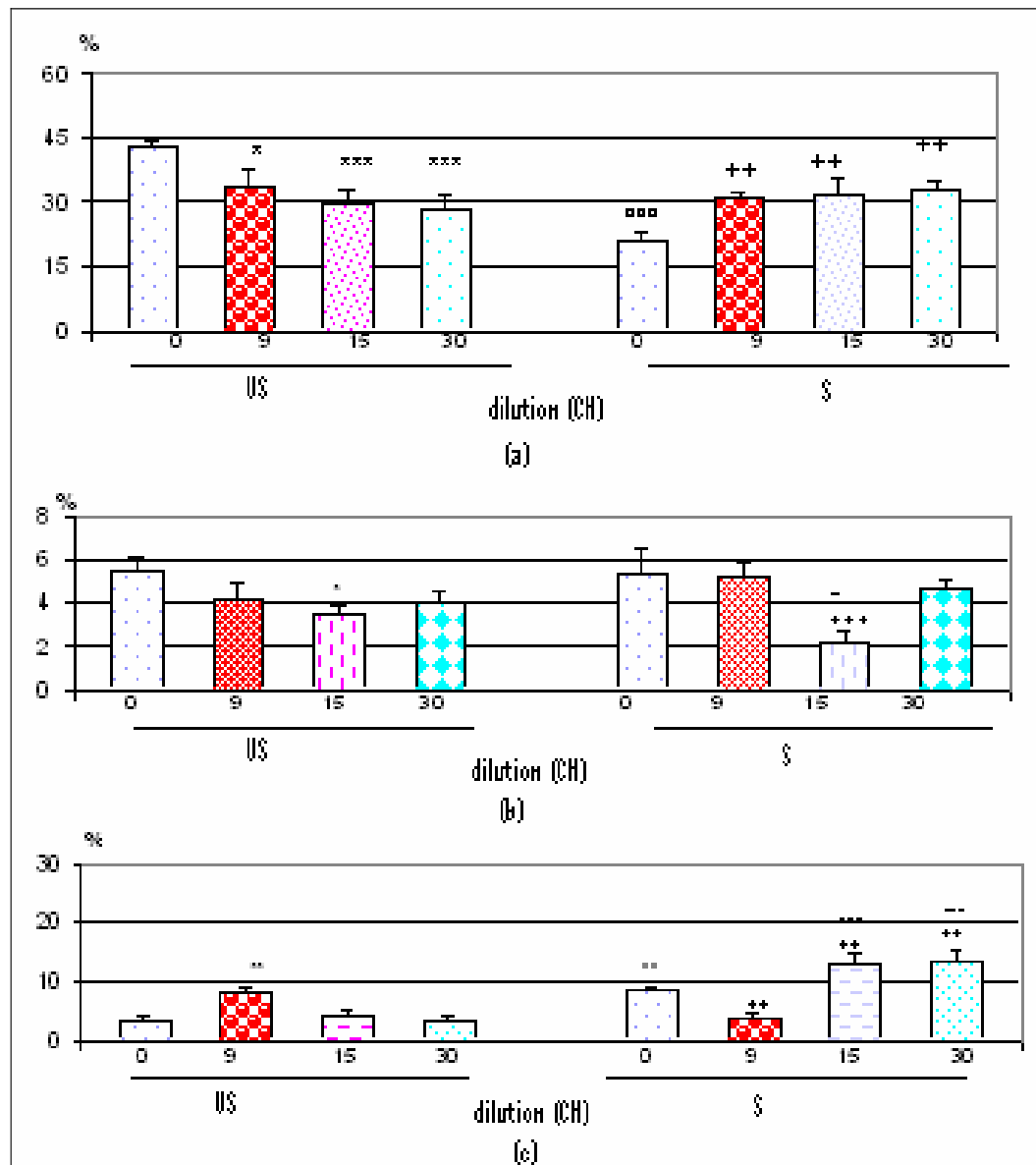


Figure 3. Effects of high dilutions of Poumon histamine on percentage of TCD4 (a), TCD8 (b) and NK (c) cells in mice. N=216. US: Unstressed. S: Stressed. CH: Centesimal Hahnemannie. (a) °: US0/S0, *: US0/USPhis9, 15, 30 CH, +: S0/SP.his9, 15 et 30. (b) *: US0/USP.his15, +: US0/USP.his15; -: USP.his15/SP.his 15. (c): °: US0/S0, *: US0/US P.his9, +: S0/SP.his9, 15, 30,-: USP.his 15, 30/SP.his15, 30.

As shown in figure 4c, high dilutions of Histaminum (15 and 30 CH) induced a significant rise of NK cells number at the same time in unstressed groups (respectively: $p=0.0047$; $p=0.01$) and stressed groups (respectively $p=0.03$;

$p=0.028$), whereas, the 9 CH of Histaminum caused rather a reduction in NK cells number in stressed animals ($p=0.004$). We supposed that this dilution of Histaminum has tendency to reverse the stress effects on NK cells.

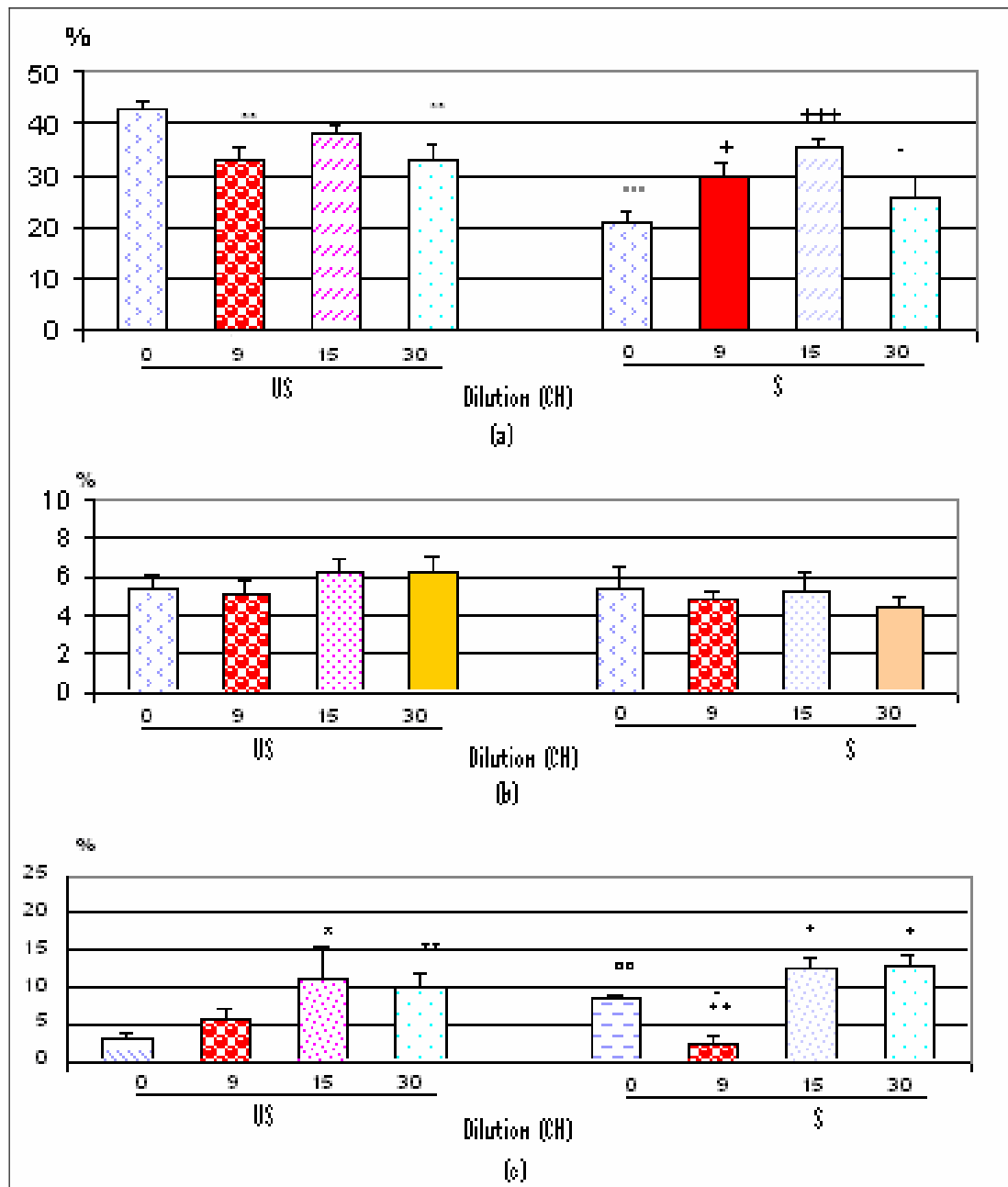


Figure 4. Effects of high dilutions of Histaminum on percentage of TCD4 (a), TCD8 (b) and NK (c) cells in mice. N=216. US: Unstressed. S: stressed. CH: Centesimal Hahnemanienne. (a): °US0/S0, *: US0/USHis9 et30,S0; +: S0/SHis9 et15; -: USHis30/SHis30. (c): °: US0/S0, *: US/USHis15, 30; +: S0/SHis9, 15, 30, -: USHis9/SHis9, +: S0/SHis9, 15, 30; -: USHis9/SHis9.

Discussions and conclusions

We noted that few works were interested to study the effects of homeopathic drugs on the immune response in stressed mice. However, their interest does not target, as in our study,

particularly the cellular immunity (TCD4, TCD8, and NK cells), but the mechanism of allergic reaction, on modulation of activation of human basophiles degranulation (Demarque *et*

al., 1995) and the modulation of immune response in its totality (Boustia *et al.*, 2001).

This study reported for the first time of the literature that ultra high dilutions of *G. sempervirens*, Poumon histamine and Histaminum would have immunomodulator effects in stressed mice. We have noted that treatment with 9 CH of *G. sempervirens*, P. histamine and Histaminum was able to reverse the immunosuppressive effects of stress on NK cells response. These results demonstrated that low dilutions, particularly 9 CH of *G. sempervirens*, P. histamine and Histaminum showed a significant immunoprotective like effect on cellular immune response in stressed mice, particularly on natural killer cells. Whereas the high dilutions of these three drugs (15 and 30 CH) showed a significant immunostimulant like effect on regulation of NK cells in stressed mice. In accordance with studies of Benshop *et al.* (1994, 1997) and in the same manner as stress, this immunostimulant effect on regulation of NK cells, could be explained by a mobilization of NK cells into peripheral circulation by activation of the β adrenergic receptors and detachment of this cellular category of the vascular endothelium; which generates their

increase in peripheral circulation. However no statistically significant variation was noted for regulation of TCD4 number in stressed-treated one compared to unstressed-treated group with all dilutions of three drugs. Concerning TCD8 cells, the results showed that high dilutions of P. histamine (15 CH) and *G. sempervirens* at 15 and 30 CH reduced rather the number of this category of cells in stressed mice.

By studying cellular immune response under stress exposure and various homeopathic drugs, we described an immunostimulating effect on regulation of NK cells for all drugs, particularly at high dilutions 15 and 30 CH in stressed group. This finding correlated probably and partly to activation of adrenergic system under stress conditions. Consequently, we can propose *G. sempervirens*, P. histamine and Histaminum at high dilutions for a possible application in immune or in some cancer diseases. However, we observed that low dilutions have rather an immunoprotective action on regulation of NK cells, which suggests a possible application as "immunoprotective-anti-stress" substance.

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